GenoSensor Corporation

GenoExplorer™ miRNA Labeling Kit

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User Manual

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Literature Citation
When describing a procedure for publication using these products, we would appreciate that you refer to them as the GenoExplorer™ miRNA Labeling Kit and the GenoExplorer™ miRNA Biochips Kit.

Patents and Trademarks
GenoExplorer is a trademark of GenoSensor. The GenoExplorer™ miRNA Labeling Kit and the GenoExplorer™ miRNA Biochips Kit are covered by patents pending.

Product Overview

The GenoExplorer™ miRNA Labeling Kit

Selective and Uniform Labeling

GenoExplorer™'s labeling system employs a 5'end ligation approach. This offers greater selectivity in the labeling of RNA compared with more traditional 3' approaches since RNA molecules with capped 5' ends (*e.g.*, intact mRNA) will not be labeled. The ligation reaction also ensures that the labeling is uniform in that a single label is attached to each RNA molecule with an uncapped 5'end. GenoExplorer™ currently offers a biotin label.

Simplified Sample Prep Protocol

With GenoExplorer™'s more selective labeling system, RNA samples do not require the additional miRNA isolation step as some labeling protocols recommend. Users can begin their labeling reaction with total RNA. It is important, however, to ensure that small RNA is retained during the initial RNA extraction procedure.

Streptavidin-Dye Staining

The GenoExplorer™ full kit also includes a streptavidin-dye conjugate which is used for the post-hybridization dye staining step. This step is required for RNA labeled with biotin.

Kit Components and Storage Conditions

GenoExplorer™ miRNA Full Kit (20 reactions)

Components	Amount	Storage
Buffer L	140 µl	-20º C
Enzyme L	10 µl	-20º C
SA-S Dye	10 µl	-20º C
Buffer S	2 x 1.4 ml	-20º C
User's Manual	1	

Shipping and Storage

GenoExplorer™ miRNA Labeling Kit reagents are shipped on dry ice. Components should be stored at temperatures shown in the above table. At proper storage conditions, components are stable for 1 year from the date received. Expiration dates are also noted on the tube labels of each component.

Safety Warnings and Precautions

For research use only. Not recommended or intended for the diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals. Consider all chemicals as potentially hazardous. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products. Wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Exercise caution to avoid contact with skin or eyes: if contact should occur, wash immediately with water (Material Safety Data Sheet for products is available upon request).

Additional Required Materials

Recommended: GenoExplorer™ miRNA Biochips kit
Recommended: GenoExplorer™ miRNA probe set
Recommended: GenoExplorer™ reagents for hybridization assay
Total RNA containing the small RNA
RNase-free water
Adjustable pipettors
RNase-free tips
RNase-free polypropylene microcentrifuge tubes (0.2, 0.5 or 1.5 ml)
Graduated cylinder
Microcentrifuge
Incubator set at 37º C
Incubator or heating block set at 75º
Heating block at 95º C
Hybridization Station (optional), for automated hybridization (*e.g.*, Genomic Solutions, Tecan) **or**
Coverslips, for manual hybridization (*e.g.*, Erie LifterSlips™ , Product # 22x30I-2-4374)
Slide Chamber, for manual hybridization (*e.g.*, Corning® Microarray Hybridization Chamber, Product #2551)
Incubator or water bath set at 42º C, for manual hybridization
50 ml screw-top tubes, for manual post-hybridization and post-stain washing
Bottles for storing diluted Wash Buffers
Centrifuge or air blower
Microarray scanner and Image Processing Software

Related Products from GenoSensor

GenoExplorer™ microRNA Full Kit
GenoExplorer™ microRNA Biochips Kit
GenoExplorer™ microRNA Probe Set
GenoExplorer™ Reagents for Hybridization Assay

GenoExplorer™ Labeling Protocol

General Description

The GenoExplorer™ miRNA Labeling Kit (patent pending) provides a direct end-labeling method. Biotin labels are ligated to the 5' ends of RNA molecules which do not contain a 5'-capped structure. These RNA molecules include rRNA, tRNAs, regulatory small RNAs such as microRNA, siRNA, snRNAs, and other RNA transcripts of yet unknown function. Due to labeling selectivity, this method has resulted in low false positive hybridization signals that are usually caused by mRNA, which are the most highly complex sequences of genome transcripts. This protocol uses directly isolated RNA without RNA target amplification, and ultimately reflects the cellular microRNA molar ratio, thus providing for the reliability of hybridization signals. The streptavidin-conjugated dye stain (SA-S) and the staining buffer (Buffer S) reagents are also included in this kit. Their preparation and use in the post-hybridization staining procedure is described below in the hybridization assay section.

Handling RNA Samples

When working with RNA, always use proper microbiological aseptic techniques. Use RNase- and DNase-free reagents, water, glassware and plasticware. Use non-powdered gloves during all steps of sample labeling, chip hybridization, washing, detection, and scanning.

RNA Preparation

The GenoExplorer™ miRNA Labeling Kit (patent pending) provides an easy and quick way to label microRNAs and other small RNAs. Total RNA isolation (not provided) using traditional methods such as Trizol is recommended. Some commercial kits can be used. Users should be aware of the harvest efficiency for small RNAs when choosing them. Checking with manufacturers is highly recommended. A total RNA starting amount of 5 to 10 micrograms is recommended.

High quality and sufficient amounts of RNA samples is crucial for experiments with microarrays. RNA quality can be evaluated by visualizing the RNA on a gel, as well as by calculating the A_{260}/A_{280} ratio. On a denaturing gel (or on an ordinary agarose gel in denaturing buffer) the RNA should appear as two bright distinct bands that represent the 28S and 18S ribosomal species. The 28S band should be brighter than the 18S band. Tailing of these major bands down the gel, or a background smear behind these bands that gets heavier at lower molecular weights can indicate degradation of the RNA. Degraded RNA will produce high background and low signal intensity microarray results.

miRNA Labeling Procedure

This procedure is used to attach a biotin label to the 5' ends of RNA molecules.

1. Place Buffer L on ice and thaw for 15-20 minutes. Check for any precipitate. If necessary, warm the solution at 37°C and agitate to dissolve the precipitate completely.
2. Mix Buffer L by vortexing followed by brief centrifugation.
3. Combine reagents according to table 1 below. Reagents should be combined in an RNAase-free microcentrifuge tube and all reagents should be kept on ice during set up of the reaction. For high accuracy, pipet the viscous Buffer L slowly.

Table 1: miRNA Labeling Reaction Mix

Reagents	**Volume (µl)**
Buffer L	7.0
RNA (5-10µg)	Adjustable
RNAase-free water	Adjustable
Enzyme L	0.5
Total	**20**

4. Mix reaction thoroughly by pipetting up and down or flicking tube gently several times.
5. Incubate at 37º C for 3 hours.
6. Incubate at 75º C for 20 min to inactivate the enzyme.
7. Store on ice until ready for the hybridization step. The labeled sample can also be stored at -70º C and used later.

GenoExplorer™ Labeled miRNA Hybridization Assay Guidelines

The GenoExplorer™ miRNA Labeling Kit has been optimized for microarray experiments using GenoExplorer™ microRNA chips and assay reagents. If microarrays other than GenoExplorer™ microRNA chips are used, consult the optimized protocols established by the microarray/probe set provider. However, it is critical to also include the post-hybridization staining step. Below are general guidelines that can be used for running the hybridization assay using GenoExplorer™ miRNA labeled samples. For a detailed protocol on running GenoExplorer™ labeled miRNA on GenoExplorer™ BioChips, please consult the user manual for the GenoExplorer™ full kit.

1. Prepare labeled RNA in hybridization buffer following the optimized protocol established by microarray/probe set provider.
2. Incubate the sample at 90-95º C for 5 min.
3. Cool sample by spinning down for 2 min.
4. Hybridize samples on microarrays following the optimized protocols established by the microarray and/or probe set provider.
5. After completion of the hybridization, slides should be washed and dried according to optimized protocols established by the microarray and/or probe set provider. If hybridization is performed in an automated hybstation, the drying step is probably not required.
6. Within 15 minutes of beginning the post-hybridization staining, the SA-S dye should be diluted 500-fold into Buffer S. For a single sample this dilution should be performed by adding 0.5 µl of the SA-S to 249.5 µl of buffer S followed by gentle mixing and brief centrifugation. Keep protected from light to minimize photobleaching of dye.
7. Load the 1:500 diluted SA-S dye stain solution on to the array and incubate at 25ºC for 30 min. Shield the slide from light to minimize effects of photobleaching of the dye.
8. After completion of the post-hybridization staining step, slides should be washed and dried according to the optimized protocols established by the microarray and/or probe set provider.

Scanning, Detection and Image Analysis

Consult the scanner manufacturer instructions for laser and PMT settings. The SA-S dye stain signal can be detected using the scanner's 635 nm wavelength (Cy5) channel. Consult the .gal file provided by the microarray manufacturer for further data analysis.

Appendix

Troubleshooting Guide

Poor Hybridization Signal

Poor quality RNA samples	• Use higher quality RNA samples • Use proper laboratory techniques when handling RNA samples • Label and hybridize more sample to microarray
Suboptimal amount of sample applied to microarray	• Label and hybridize more sample to microarray
Improper detection strategy	• Verify that detection instrumentation is compatible with detection reagents • Adjust detection settings
Signal lost by exposure to light, environmental conditions	• Minimize exposure to light
Poor biotin/ SA-S dye detection	• Ensure that the samples have been purified and quantified properly before labeling • Ensure that the kit components have been stored properly • Ensure that the detection solution was prepared and stored properly
Hybridization signal “stripped” from microarray	• Ensure correct temperatures are used for each of the process steps.
Suboptimal hybridization time	• Extend hybridization time

High Background

Excess detection reagents remaining on microarray	• Do not allow slides to dry out during hybridization protocol • Do not touch microarray directly or forcibly remove coverslip at any time
Excess sample applied to microarray	• Quantify the amount of labeled sample and use less in hybridization
Ink or marker used to identify microarray	• Avoid using markers or ink to identify slide; use a diamond scribe pen

Dust

Dust on microarray	• Avoid dust from environment • Minimize exposure of the microarray to the air • Use filtered distilled water for final wash

Technical Service

For more information or technical assistance, please call, write, fax, or email.

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Limited Warranty

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